

PATENT/OFFICIAL

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Fatima FERREIRA et al.
Title: ALLERGEN FROM MUGWORT POLLEN
Appl. No.: 10/517,052
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Examiner: Nora Maureen ROONEY
Art Unit: 1644
Atty Docket No: LNK-036
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Mail Stop AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. 1.132

Sir:

I, Professor Fatima FERREIRA, hereby declare and say that:

1. I am a citizen of Austria.
2. I am a joint inventor of the subject matter presently claimed in the above-captioned application.
3. I have reviewed the Office Action issued August 1, 2007 in connection with the above-captioned application, including the allegedly anticipating disclosures cited by the Examiner, namely the publications by Nilsen et al. (Mol. Immunol., 28(7):733-742, 1991), Brandys et al. (Planta. Med., 59: 221-228, 1993),

Hirschwehr et al. (J. Allergy Clin. Immunol., 101(2): 196-206, 1998), de la Hoz et al. (Mol. Immunol., 27(7): 651-657, 1990), Katial et al. (Annals of Allergy, Asthma & Immun., 79: 340-346, 1997), and Paulsen et al. (Int. Archs. Allergy Appl. Immun., 78:206-212, 1985), as well as Gen Bank Accession Number AY904433.

4. Having reviewed the above-referenced publications, I submit that none of the “approximately 44 kDa polypeptide allergens isolated from mugwort pollen using SDS-PAGE gel” allegedly described by Nilsen et al., Brandys et al., Hirschwehr et al., de la Hoz et al., Katial et al. or Paulsen et al. is identical to the presently claimed polypeptide of SEQ ID NO: 1, referred to in Gen Bank Accession Number AY904433 as “Art v 6”.
5. The Examiner is correct that the amino acid sequence set forth in SEQ ID NO: 1 of the instant application corresponds to the Art v 6 protein of Gen Bank Accession Number AY904433. The deduced amino acid sequences for the Art v 6 signal peptide and mature protein are set forth in Appendix A attached hereto. The calculated molecular weight for the Art v 6 protein is 40,834.55 Daltons (noted as ~40.9 kDa in the Examples of the instant application) and the theoretical isoelectric point (pI) is 8.27
6. With respect to the above-referenced Nilsen publication, although Nilsen et al. identified at least 15 IgE-binding components with molecular weights ranging from 12 kDa to 100 kDa, the most prominent of which had molecular weights of 12.5, 22, and 63 kDa, the authors provide no sequence information that would unequivocally prove that any of the isolated polypeptides is indeed identical to Art v 6. Moreover, none appear to have a molecular weight of 40,834.55 Daltons (see Figure 1 and Table 1) or a theoretical isoelectric point (pI) of 8.27 (see Figures 2-4). Accordingly, I submit that none of the allergen polypeptides described by Nilsen et al. is identical to the presently claimed ~40.9 kDa Art v 6 protein defined in SEQ ID NO: 1.

7. With respect to the above-referenced Brandys publication, while in the course of investigating the allergenic cross-reactivity among six mugwort (*Artemisia*) species, Brandys et al. observed similar band patterns for all extracts, especially in the molecular weight region of 25 kDa to 90 kDa, the authors provide no sequence information that would unequivocally prove that any of the isolated polypeptides is indeed identical to Art v 6. Moreover, none appear to have a molecular weight of 40,834.55 Daltons (see Figure 2) or a theoretical isoelectric point (pI) of 8.27 (see Figure 3). Accordingly, I submit that none of the allergen polypeptides described by Brandys et al. is identical to the presently claimed ~40.9 kDa Art v 6 protein defined in SEQ ID NO: 1.
8. With respect to the above-referenced Hirschwehr publication, while Hirschwehr et al. identified a number of allergenic structures common in mugwort and ragweed pollen, the authors provide no sequence information that would unequivocally prove that any of the isolated polypeptides is indeed identical to Art v 6. Moreover, none appear to have a molecular weight of 40,834.55 Daltons (see Figures 1-3) or a theoretical isoelectric point (pI) of 8.27 (not shown). Accordingly, I submit that none of the allergen polypeptides described by Hirschwehr et al. is identical to the presently claimed ~40.9 kDa Art v 6 protein defined in SEQ ID NO: 1.
9. With respect to the above-referenced de la Hoz publication, de la Hoz et al. describe the purification of a mugwort allergen which was incorrectly designated as "Art v 1". However, the authors provide no sequence information that would unequivocally prove that the isolated polypeptide is indeed identical to Art v 6. Furthermore, since under native conditions the protein was estimated to be 47,000 Da and under denaturing (SDS-PAGE) it was estimated to be 60,000 Da, the real molecular weight of the purified "Art v 1" protein is not known. In contrast, Art v 6 has a calculated molecular weight of 40,834.55 Da and under denaturing conditions (SDS-PAGE) it migrates as a 40,000 Da protein (see Figure 5 of the

instant application). In addition, while analytical isoelectric focusing showed that the protein isolated by de la Hoz et al. is an acidic protein having a pI of 4.4, Art v 6 is a basic protein with a pI of 8.2. Accordingly, I submit that the "Art v 1" protein described by de la Hoz is not identical to the presently claimed ~40.9 kDa Art v 6 protein defined in SEQ ID NO: 1.

10. With respect to the above-referenced Katial publication, Katial et al. utilized ELISA and IgE immunoblots to investigate cross-reactivity among mugwort (*Artemisia*) species. However, the authors provide no sequence information that would unequivocally prove that any of the isolated polypeptides is indeed identical to Art v 6. In addition, while Katial et al. describe IgE-binding peptides in all extracts in the 66-kDa, 45-kDa, and 21-kDa ranges, they fail to specifically mention an IgE-binding protein in the range of 40-kDa. Accordingly, I submit that none of the allergen polypeptides described by Katial et al. is identical to the presently claimed ~40.9 kDa Art v 6 protein defined in SEQ ID NO: 1.
11. With respect to the above-referenced Paulsen publication, Paulsen et al. describe the extraction of allergens from mugwort pollen using two different buffer systems, phosphate-buffered saline or NH_4HCO_3 . Gel filtration of the NH_4HCO_3 extract indicated the presence of proteins in the MW region of 20,000-70,000. The extract represents a complex mixture of different proteins. Since no further purification of the individual components and IgE-binding tests were performed, it cannot be said with any certainty whether the disclosed components are allergenic. In any event, the amino acid analysis of Paulsen's crude extract yields an amino acid composition that is quite distinct from that of Art v 6 (see the amino acid content comparison set forth in Appendix B, attached hereto). Accordingly, I submit that none of the polypeptides described by Paulsen et al. is identical to the presently claimed ~40.9 kDa Art v 6 protein defined in SEQ ID NO: 1.

12. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

Respectfully submitted,

20.11.2007

Date

Fatima Ferreira

Professor Fatima FERREIRA

APPENDIX A:

Deduced amino acid sequence of Art v 6:

MEKH YFVILFTAA FVFVGAAARADIGDELEAAQFNSTRRLHECAAHNIIDKCWRCKAD
WEKNRQALAKCAQGFAGTTGGLGGEIYVVTDCSDDNAANPKPGTLRCGVTQDKPLWII
FKKDMVIK LKHELVINKDKTIDGRGANVEITCGGLTIHNV CNV I IHN IHI HDIKVTEGG
I I KATDAKPGHRHKSDGDGICVAGSSKIWIDHCTLSHGPDGLIDVTLGSTAVTISNCKF
SHHQKILL LGADNSHVDDKKMHVTVAFNRF AEACDQRMPCRFGFFQV VNNDYTSWGTY
AIGGSANPTILSQGNRFHAPNDPMKKNVLVRANAPHPE SMKWNWRSEKD LLENGAIFVA
SGCDPHLTPEQKSHLIPAEPGSAVLQLTSCAGTLKCVPGKPC

Red: signal peptide

Black: mature protein

APPENDIX B:

The amino acid composition
of Art v 6 (SEQ ID NO: 1)

Ala (A)	31	8.3%
Arg (R)	15	4.0%
Asn (N)	22	5.9%
Asp (D)	26	6.9%
Cys (C)	17	4.5%
Gln (Q)	10	2.7%
Glu (E)	14	3.7%
Gly (G)	34	9.1%
His (H)	19	5.1%
Ile (I)	28	7.5%
Leu (L)	24	6.4%
Lys (K)	29	7.7%
Met (M)	5	1.3%
Phe (F)	11	2.9%
Pro (P)	17	4.5%
Ser (S)	19	5.1%
Thr (T)	22	5.9%
Trp (W)	7	1.9%
Tyr (Y)	3	0.8%
Val (V)	22	5.9%
Hyp		0.0%

The amino acid
composition of mugwort
pollen extract containing
proteins with MW ranging
from 20-70kDa

Ala (A)	9.9%
Arg (R)	0.7%
Asn(N) /Asp (D)	11.3%
Cys (C)	1.4%
Gln(Q) /Glu (E)	9.1%
Gly (G)	11.8%
His (H)	2.0%
Ile (I)	3.6%
Leu (L)	5.7%
Lys (K)	8.4%
Met (M)	1.3%
Phe (F)	3.7%
Pro (P)	4.6%
Ser (S)	5.1%
Thr (T)	5.9%
Trp (W)	1.9%
Tyr (Y)	2.4%
Val (V)	5.9%
Hyp	4.4%